In re the Application of:

BA HELM, APM WILSON, D MOREIRA-MACHADO, CE PULLAR and A CAMP (assigned to EURO DPC)

Serial no: 09/133,766 (Continuation)

Filed: 8 December 1998 as a continuation of USSN, 08/446,760 filed 25 November 1993

Title: Allergen/Inflammatory Testing & Diagnosis

Signed: A P Wilson

Dated: Originally dated 4 November 2003, resubmitted July 2004

DECLARATION UNDER 37 CFR 1.132

I, A Penelope Wilson declare that my credentials are as listed in the attached curriculum vitae, for which my references are available on request;

I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

I understand that the above patent application, of which I am an inventor is pending before the US Patent and Trademark Office and that a rejection to Claims 44 and 47-54 remains outstanding.

The nature of the Rejection

Claims 44 and 47-54 have been rejected under 35 USC 112 first paragraph as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention", ie the rejected Claims relate to a method for which the specification is not enabling.

The Examiner has based his rejection on *Benyon et al*, which he considers teaches that a variety of molecules which are not allergens (ie compound 48/80, poly-L-lysine, substance P, VIP, somatostatin) cause the release of the mediator histamine from mast cells. ***The Examiner has stated that these substances are not allergens. Thus based on the teachings of Benyon et al., the Examiner has objected that histamine release in itself does not indicate that a substance will also cause IgE mediated allergic reactions.***

The Examiner has noted that in fact that *Benyon et al* refers to release of histamine by mast cells in response to the aforementioned substances as caused by "non-immunological stimuli". ***The Examiner notes that while Benyon et al. also disclose that specific different specific mediators are released by IgE versus IgE independent mast cell activation, the instant claims do not recite release of any particular mediator. He states that thus the claimed invention could not be used to determine the allergenicity of a substance because nonallergens also cause the release of mediators from mast cells.***

The Examiner states that in fact *Benyon et al* disclose that nonallergens can cause the release of histamine from mast cells in similar amounts to that seen when allergens are used in the screening assay. The claimed invention encompasses a method wherein histamine is the only mediator assayed.

Finally the Examiner has stated that if the agent causes release of mediators from mast cells in the absence of IgE, using the screening assays disclosed in the specification it would not be possible to determine if the agent was an

allergen per se (eg capable of inducing IgE antibodies) because the agent causes release of mediators from mast cells in the absence of IgE. ***Therefore that the specification is not enabling for the claimed invention.***

The Examiner accepts the novelty and inventive step of Claims on file.

The basis of this Declaration

In order to show that the specification is enabling for the claimed method I hereby declare, and give detail hereinafter, for the following:

- ***Nonallergens: I hereinbelow declare that non-immunological "secretagogues" (compound 48/80, poly-L-lysine, substance P, VIP, somatostatin) are potentially allergenic, and indeed some of these cause symptoms which have the hallmark of an allergic response in addition to having IgE adjuvant activity and would cause a direct or IgE adjuvant mediated allergic response. Therefore mediator release by non immunological secretagogues (Benyon et al) does not contradict the method of the invention, since such substances which the Examiner has termed "non-allergens" have the potential of steering immune responses to bystander antigens, i.e. several mast cell secretagogues induce the release of substances capable of creating a microenvironment which favours the subsequent induction of an IgE isotype.***

- ***Controlled Screening Assay: I also declare that the method of the invention relates to an assay, as defined in the amended Claim 44, and that assays of the invention were conducted utilising the appropriate negative and positive controls and reference materials. Known allergens gave rise to mediator release whilst inactivated allergen preparations served as negative controls. In addition a number of mediators were assayed which verified the results and demonstrated that the invention is enabled for more than just histamine release.***

- ***The Basis of Screening Assay methods: I also declare that the present invention relates to a method for screening assay of unidentified "virgin" substances the allergenicity of which is not known, to determine allergenicity without the addition of IgE, and should therefore be considered as a first screen. The invention is therefore enabled as the skilled artisan would appreciate that such results may require verification or confirmation. Moreover we are able to tell the difference between an IgE and non-IgE response by the shape of the dose response curve. Furthermore one could look at the direct binding of the potential allergen to specific IgE (ie screen serum as I describe below).***

- ***Non-immunological stimuli capable of causing immunological release: I also declare that the method of the invention is based on the sound findings of our research and more recent research of others which shows that long term or downstream effects of mediator release can cause IgE synthesis, leading to immunological release.***

The detail of this Declaration

Nonallergens

Previous Statements

We have previously submitted in our response dated 16 December 2002 that mediator release by non-immunological secretagogues (*Benyon et al*) does not contradict the method of the invention, since such substances are also capable of steering subsequent immune responses towards an allergy. The Examiner has rejected this submission, in the form of various statements regarding what is known in the art and experimental results that have been obtained, under MPEP Section 716.01 © (Rev. 1 Feb 2003) as Attorney arguments which cannot take the place of evidence.

The rejected statements are my own statements and are based on my extensive experience in this field. I hereby resubmit those statements as follows:
Benyon et al in fact support the viability of this invention. The method of our invention is looking for substances which cause non-immunological mediator release and therefore *Benyon et al's* results support the present invention, although *Benyon et al* did not recognise the significance of their observation in relation to potential allergenicity, since the molecular mechanisms leading to the development of allergic responses were less well defined at the time their observation was made. *Benyon et al* deduced that the mediator release was due to an incidental factor such as the closeness of some mast cell populations to nerve endings. In fact the skilled person would not at the time, on reading *Benyon et al*, have appreciated that the release observed was an indication of potential allergenicity, either directly or mediated by an IgE adjuvant activity.

The skilled person, on reading *Benyon et al* and being equipped with the knowledge and understanding which has been built up since 1989 by progress in this field, but particularly in view of the knowledge built up from my and my co-inventors own work leading to our invention, would appreciate that *Benyon et al* had in fact observed non-IgE mediated release, which he has termed non-immunological, and which may be linked to potential allergic responses in individuals. Our invention therefore shows that substances have been observed in the past, for example by *Benyon et al*, to induce release of then-unrecognised mediators, which have since been classified as mediators, and the substances classified as allergens whose effect is direct or is mediated by IgE adjuvant activity.

In fact there are now several hundred such mediators including isoforms, and there could well be more mediators or pro-allergenic mechanism, which await identification. In our method, and this was initially the surprising observation, the mediators released by the potential allergens and environmental pollutants which we investigated, like cigarette smoke or diesel exhaust particles, which

have IgE adjuvant activity to bystander antigens, which are now in the absence of IgE sensitisation are the same as those released as a result of an IgE-mediated antigenic stimulus. Although we did not look at enough mediators to say that they are the same, and indeed *Levi-Schaffer et al* have investigated differences, we were able to show that at least some of the same mediators are released by at least some of the same substances.

It is now known that people can get stress or heat or cold induced asthma attacks, allergies such as sensitivity to cigarette smoke or diesel exhaust particles etc., which are not IgE mediated, and it is therefore possible to get the incredible cascade of mediators leading to anaphylactic shock. It is also known that there are very potent substances found within the human body, which substances can cause release *in vivo* and *in vitro*. The present invention in fact aims to detect potential allergens, which are found outside the human body. However the present invention also has the potential of detecting and assessing mast cell activation by endogenous substances. It is known that a mammalian organism in response to external stimuli such as stress produces such substances. It is now known that endogenous cellular mediators, released by activated mast cells (e.g. mast cell proteases) or triggered eosinophils (e.g. rantes) induce mast cell degranulation and cause symptoms of allergy via a non-IgE-mediated mechanism. The present invention represents the first instance that this was appreciated.

Benyon et al in fact investigated substances, which caused release in the human body, and which correspond to the sort of substances, which we would be looking for, using the method of our invention.

New statements

My coinventor Helm was aware of the *Benyon et al* reference at the filing date of this application. Indeed other papers exist and predate *Benyon et al* and

disclose a vast number of substances capable of triggering mast cell release in the absence of sensitisation, many of which are in fact allergens.

Benyon et al does not state that the subject non-immunological "secretagogues" are not allergens. At page 898 column 1 he states that "skin mast cells may be activated *in vivo* by cross-linkage of their IgE-receptors. However this is not the only mechanism by which these cells may be activated.... skin mast cells secrete histamine in response to a variety of non-immunological secretagogues including compound 48/80, poly-L-lysine, substance P, vasoactive intestinal peptide (VIP) and somatostatin". He reports that the IgE dependent and non-immunological stimuli differ in their capacity to activate release of eicosanoids and suggests that the two stimuli use different secretory mechanisms. He states at page 902 column 2 that "the physiological implications of these findings are uncertain but are worthy of consideration... immunological and non-immunological stimuli may have bi-functional roles in the dermis." *Benyon et al* goes on to speculate a homeostatic function of non-immunological stimuli such as neuropeptides.

In my opinion were the method of our invention performed with exposure of cells as described to the non-immunological substances of *Benyon et al*, the method would result in mediator release and a determination that these substances are potential allergens whose effect is either direct or is mediated by an IgE adjuvant activity. This would support my opinion that the substances may indeed be potential allergens, but in fact as many are extremely powerful agents - some of the venoms would probably cause death before they had the chance to evoke an allergic response – their status as allergens has not been recognised *in vivo*. Indeed scorpion venoms are related to bee/wasp/hornet allergens but are so powerful that they could, as a toxin, kill an individual, before initiating an allergic response. However, it is noteworthy that classical allergic responses, and indeed death from anaphylactic reactions in response to repeated scorpion stings have been recorded. The skilled person would without

doubt treat these potent substances with care and avoid direct contact. Accordingly the classification of such secretagogues by the Examiner as non-allergens is unfounded and is certainly not established in the literature. I therefore consider that the Examiner has based his objection on an unfounded premise, and that the factual verification of this is yet to be established in the art.

It is well known that some substances do not cause mediator release in low doses but above a certain dose can cause release, and this further supports the idea that self proteins may not elicit an allergic response in low dose *in vivo* but may steer subsequent allergic responses to bystander antigens if presented to a subject or to mast cells in higher doses or if presented differently (ie could have problems with mast cells when inhaled).

The Examiner has noted that Benyon has in fact determined that specific different specific mediators are released by immunological and non-immunological stimuli. In my opinion this is irrelevant to the matter in hand. The art (*Levi-Schaffer et al, Komisar et al* previously referred) documents different release mechanisms, different release levels, different release promoters and so on, and it is clear that there is much diversity in responses to different substances, presented in different doses, singly or repeatedly presented etc. This is borne out by the clinical observation that each allergy and each exposure incident may be different from the next in severity or response, onset of allergy and disappearance of allergy in older or younger subjects and the like.

In fact in the method of the invention the cells could potentially release any mediator, to a greater or a lesser extent, with which it was preloaded by virtue of the degranulation event caused by a potential allergen or e.g. environmental pollutants with IgE adjuvant activity.

Controlled Screening Assay

New statements

I draw the Examiners attention to the fact that, as mentioned above, the present application includes examples in the form of methods which have been conducted with appropriate controls, in order to eliminate the instance of such misinterpretation of results. I refer the Examiner to Example 1 at Page 15 lines 6 to 13 and Example 2 at page 16 lines 16 to 21 which provide an Example of incubating transfected cells which had been preloaded with mediator, with serum from an individual known to be sensitised to bee venom, and challenging with bee venom. A dose response curve was obtained as shown in Figure 3 and Figure 4, confirming that release of mediators (5-HT) is an indication of IgE mediated allergic reaction in a sensitised individual. This example acts as a reference for Example 2 at page 16 lines 21 to 25, in which transfected cells preloaded with mediator (5-HT) were challenged with bee venom in the absence of sensitising serum and showed release of mediator 5-HT.

Significantly the Figure 4 shows a different relation of dose and response for sensitised and non sensitised cells, notably a classical "allergic" "bell shaped response with increasing doses of venom, for sensitised cells, and an increasing response with increasing doses of venom for non-sensitised cells. We respectfully submit that the observations of Benyon *et al* are not inconsistent with the method of the invention, since both show that the levels of mediator released by non-immunological stimuli may differ from those of immunological stimuli but the mediators themselves remain a valid indication of potential allergenicity.

Finally in Example 2 at page 16 line 26 to page 17 line 4 and Table 2, the results of challenging with other test substances are shown, and again these are

verified by the above Control examples, indicating that mediator release is an indication of potential allergenicity.

The skilled artisan would appreciate that a negative control may be performed by mutating a potential allergen in a point mutation whereby it retains its overall configuration but is deactivated in terms of activating mediator release. We were the first to report this for the allergenicity of enzymatically active bee venom phosholipase A and not its enzymatically inactive counterpart ("A link between Catalytic Activity, IgE-independent mast cell activation, and Allergenicity of Bee Venom Phospholipase A2" Dudler et al, J. Immunol, 1995, 155:2605-2613). We also conducted just this control which we reported in our paper "Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE", Machado et al Eur J Immunol, 1996, 26: 2972-2980, in which we reported that an essentially inactivated house dust mite preparation caused no release. I refer the Examiner to more recent publications such as "The cysteine protease activity of the major dust mite allergen Der p1 selectively enhances the IgE antibody response", Gough et al, J Exp Med, 1999, 190(2), 1987-902.

The Table 2 of the specification shows that levels of at least two mediators (5-HT (preloaded) and protease(naturally occurring)) are released in proportional amounts for any given substance, in some instances release of a third mediator beta-hexosaminidase (naturally occurring) was also measured. The important factor in the assay method of the invention is that a series of mediators is released by a potential allergen and that anyone of the mediators could be measured as a readout. We would not expect the mediators released by non-immunological and immunological IgE mediated release to be significantly different. If degranulation occurs then it is the contents of the secretory granules that are released. The contents themselves may vary in terms of the relative amount of each mediator depending on what the cell has synthesised –

which in turn is dependent on the cellular environment and on what may or may not have been preloaded. It does not necessarily follow, and is not of importance to the determination to be drawn from the assay method, that each mediator plays a role, in vivo, in generating an IgE response to the potential allergen.

Accordingly we respectfully submit that it would be unduly limiting of the scope of the invention to restrict Claim 44 to detection of release of certain mediators only, such as exemplified in the present application.

The Basis of Screening Assays

New statements

I hereby state that the specification describing our invention is enabling for the claimed invention which is designed as a simple screening assay for determining potential allergenicity as indicated by mediator release. Screening assays are intended to simplify and ideally speed up screening of many substances simultaneously or in succession. The screen operates on the principle of a proven assay method which has usually been shown to give a positive or negative indication or other such indication or diagnosis. Screening assays typically produce less than 100% sensitivity and less than 100% specificity but give a rapid means to screen a number of candidates for a specific property or condition, and false positives are routinely eliminated as known in the art.

It is common in the art of clinical chemistry to devise a screening assay to determine a positive grouping of subjects or conditions and subject to further tests performed under more stringent conditions. The method of the invention is no different from such screening assays and is defined as determining a potential allergen and by inference, any substance with IgE-adjuvant activity which is thus capable of mediating an allergic reaction. It would be within the

knowledge of the skilled in the art to further investigate the results of the screening assay of the invention and further refine the determination obtained with the assay. We refer to Systemic reviews in health care: "Systemic reviews of evaluations of diagnostic and screening tests", Deeks, JJ. BMJ (2001) 323,157-162 "Principles of Screening" Eva, M I, Krvchenia E L, Clinics in Perinatology, 28, (2), 273-278 (2001).

A very well known screening assay is the "Ames" test for potential carcinogenicity and it is well recognised that even this highly established and well reputed test does not give 100% specificity. Similarly the well known and routinely followed triple test for Downs syndrome is known to be considerably less than 100% accurate.

The method of the invention as described in the specification is conducted with controls as described above in order to verify the results obtained and conclusions drawn. Specifically controls applied in the invention as described in the specification include a test against known positive bee venom, and a test against both fresh and auto-catalytically degraded samples of known positive dust mites, in the latter the old sample being found to be enzymatically inactive and devoid of secretagogue activity. This illustrates:
That the method can produce a positive result in a case known to be positive;
That the method can distinguish between two samples of a known allergen and indicate one sample as being inactive.

The Examiner has questioned whether the method would distinguish allergens from non-allergens. The above control clearly indicates that this is the case for enzymatically inactive dust mite emanations.

We submit that the method of the invention may be complemented with the use of further tests which confirm or reject the findings of the screening assay of

the invention, whereby the **potential** allergenic status of the substances may be determined.

Referring to the above comment on screening assays in general, it should be appreciated that a positive result in any screening assay is typically referred for further tests to verify or clarify the result obtained. In this case a further test may comprise repeating the test with detection of interleukins such as IL4 and/or IL-13, confirming that the marker detected is indeed indicative of interleukin release, which is the first stage of an allergic response. The interleukin test is very expensive and would not be realistically conducted in screening a large number of candidate allergens. Failure to verify the result may lead to repeating the test method with fresh sample or with sample prepared in different solvents for example, which would indicate if the sample was contaminated or in the case of false negative results deactivated.

Thereafter verification may comprise determining the antibody IgE specific for the detected potential allergen and screening blood or tissue samples from blood banks to search for any existing blood sample containing specific for this substance, and indicating that the allergy to that allergen already exists in one or more individuals and that the potential allergen can be upgraded to an allergen as IgE antibodies to it have been created in a human or animal. Thereafter suitable packaging notices may be amended to indicate the chemical as a potential allergen or allergen, or other precautions may be taken to avoid accidental onset of allergic reaction, or in extreme cases the substance may be classified as not for use in environmental applications such as packaging, foods and the like.

A negative result in a screening assay is also typically treated with caution as the result may arise from deactivation of a true potential allergen (see above), as in the case of house dust mite in the present invention, sampling in too low a concentration, or simply from sampling an inactive/ degraded source.

In my opinion the implications of the method of the invention is so significant that were the method of the invention to produce a false positive result, designating a true non-allergen as potentially allergenic, this would be trivial in relation to the alternative situation that the method fails to detect a true positive result, thereby failing to recognise a potential allergen. Any result may be verified or eliminated by further assays as desired, but until the role of non-immunological secretagogues art is fully established in the art these should be treated with caution and indeed treated as potential allergens.

Accordingly the teaching of Benyon *et al* infers a situation in which the method of the invention may detect a substance as potential allergen, when in fact it may prove not to be an allergen. This is not inconsistent with the claims of the application which claim the identification of a **potential** allergen. In this case however it should be appreciated that a positive result obtained with the method of the invention for substances recited in Benyon *et al* would be valid, even though these substances include self-proteins and substances found in the human body. These substances are highly potent and should be treated with great caution and would indeed cause a nasty reaction if brought into contact with mucosal membranes of most individuals.

Non-immunological stimuli are capable of immunological release
New statements

Finally the Examiner has argued that an agent which causes release of mediators from mast cells in the absence of IgE in the method of the invention is not shown to be capable of generating IgE antibodies and causing immunological release as an allergen. It is clear from the Examples and Table 2 that substances tested caused non-immunological release and are well known allergens, such as latex, bee venom etc.

It should be appreciated that IgE antibodies are known to be generated by class switching and the stimulus for class switching leads to onset of sensitisation. It is now documented in the literature that the long term or downstream indirect effects of mediator release have a part to play in triggering class switching. For example *Levi-Schaffer et al* study long term effects of mediator release, and recent work shows that prostaglandins can stimulate IgE production: "Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE secreting cells", Fedyk et al, Proc Natl Acad Sci USA 1996, 93(20), 10978-83; and "Prostaglandin E2 promotes B lymphocyte Ig isotope switching to IgE^1", Roper et al, J Immunol, 1995, 154(1) 162-70.

Although there are many ways of onset of allergic reactions including progressive exposure and sudden onset, the finding that a substance causes mediator release in the absence of IgE shows that it is capable of becoming an allergen in the future. A substance which fails to cause mediator release on the other hand has no known mechanism by which it can cause production of IgE whereby it would be classed as an allergen and capable of eliciting an allergic response.

CONCLUSION

Accordingly we submit that Benyon *et al* fails to show that the method of the invention is not enabled. Specifically we submit that:

It is not established in the art that "non-immunological secretagogues" are not allergens or potential allergens;

Appropriate controls for the method are enabled and moreover are well known in the art - both positive and negative control results have been indicated and obtained;

The need to verify results of the method would be well known to one skilled in the art of screening assays;

The basis for detecting any mediator as an indicator of potential allergenicity, either directly or mediated by IgE adjuvant activity is enabled in the screening assay of the invention *in vitro*. It does not necessarily follow, and is not of importance to the determination to be drawn from the assay method, that each mediator plays a role, in vivo, in generating an IgE response to the potential allergen.

The general state of the art

I refer the Examiner to statements made by my co-inventor Birgit A Helm, under this heading, in the Declaration dated 16 December 1999 and referencing publications of our work subsequent to this invention, illustrating the nature and diversity of allergy and the issue of **potential** allergenicity, effective directly or mediated by IgE adjuvant activity.

REFERENCES

Levi-Schaffer et al, previously referred and of record

Komisar et al, previously referred and of record

"A link between Catalytic Activity, IgE-independent mast cell activation, and Allergenicity of Bee Venom Phospholipase A2" Dudler et al, J. Immunol, 1995, 155:2605-2613 – referred to in earlier declaration of Helm 14.12.99

"Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE", Machado et al Eur J Immunol, 1996, 26: 2972-2980 – referred in earlier declaration of Helm 14.12.99

"The cysteine protease activity of the major dust mite allergen Der p1 selectively enhances the IgE antibody response", Gough et al, J Exp Med, 1999, 190(2), 1987-902

"Systemic reviews of evaluations of diagnostic and screening tests", Deeks, JJ. British Med Journal (2001) 323,157-162 "Principles of Screening" Eva, M I, Krvchenia E L, Clinics in Perinatology, 28, (2), 273-278 (2001).

"Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE secreting cells", Fedyk et al, Proc Natl Acad Sci USA 1996, 93(20), 10978-83

"Prostaglandin E2 promotes B lymphocyte Ig isotope switching to IgE[1]", Roper et al, J Immunol, 1995, 154(1) 162-70.